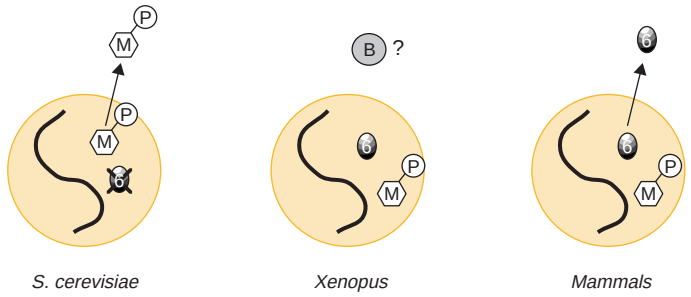

Figure 2 **Redistribution of prereplicative-complex components between nucleus and cytoplasm during S/G2 phases of the cell cycle in different organisms. *S. cerevisiae* exports Mcms and degrades Cdc6; *Xenopus* excludes another factor, possibly RLF-B (B); and mammalian cells export Cdc6. Phosphorylation (circled 'P') of Mcms may be important in their localization in *S. cerevisiae*.**

during G2 phase[9] and has potential site for phosphorylation by CDKs close to its nuclear-localization sequence[7].

Although Labib *et al.* studied yeast[1], their results have clear implications for the control of DNA replication in higher eukaryotes. Unlike yeast, metazoan Mcm proteins do not change their subcellular distribution during the cell cycle, being exclusively nuclear. Despite this difference, higher eukaryotes do seem to regulate replication licensing and the formation of pre-RCs by selectively partitioning proteins between nucleus and cytoplasm (Fig. 2). The cell cycle of the early *Xenopus* embryo has no appreciable G1 period, and the relicensing of replicated DNA occurs only in late mitosis and is absolutely dependent on the breakdown of the nuclear envelope[4]. The exclusion of an essential licensing component by the nuclear envelope would provide a simple way of preventing re-replication of DNA in a single cell cycle. It isn't known which factor is excluded, as Mcms and Cdc6 appear to remain nuclear throughout interphase, though RLF-B remains a good candidate[3].

Yet another variation is seen in mammalian cells, where Cdc6, rather than Mcms, appears to be regulated by subcellular localization[10]. In G1 phase, mammalian Cdc6 is nuclear, but it becomes cytoplasmic in S phase and remains in the cytoplasm until the following G1. Although the mechanism causing this relocalization is unknown, a role for CDKs is plausible.

Although yeast, *Xenopus* and mammals have apparently chosen different protein targets, all three seem to use exclusion of essential initiation factors from the nucleus to prevent re-replication of DNA in a single cell cycle. This physical separation of a protein from its substrate may provide a robust way to make sure that when the cell says 'no', it means it. □

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Sensor-less checkpoint activation?

Rhett Michelson and Ted Weinert

DNA damage activates a set of proteins whose job is to delay the cell cycle until the damage is repaired. This process was thought to involve the detection of damage by sensor proteins, which transmit a signal to a key protein kinase and thence to downstream targets. Unexpectedly, damage can also activate the kinase directly.

Biologists marvel, and we are all thankful, that cells maintain generally stable genomes. Genome stability, a fight against entropy, is due in large part to a host of homeostatic mechanisms, including the repair of damaged DNA. A key aspect of DNA repair involves delays in the cell cycle called checkpoints, which provide normal cells with the critical time needed for efficient repair. If cells with damaged genomes continue to replicate and divide, they invite still greater damage; for example, an innocuous gap can be converted to a threatening double-stranded break in the DNA if it is replicated before it is repaired. So checkpoints block DNA replication after damage. At the heart of checkpoints are the proteins required for cell-cycle arrest. These highly conserved proteins, found in fission and budding yeasts, flies and humans, are gradually revealing the secrets of how they function at the molecular level. On page 393 of this issue, Edwards *et al.*[1] ask how a key checkpoint protein kinase, called Rad3 in fission yeast, is activated by damage. Their findings, stitched together with observations of homologous protein kinases — human ATM and budding yeast Mec1 — allow tentative speculation about how DNA damage activates protein kinases and about the roles of other checkpoint proteins.

Why do checkpoints fascinate us just now? One possible reason is that we are learning that checkpoint proteins have even more widespread roles in regulating chromosomal events than previously thought, with some functions even being independent of cell cycling *per se*. Checkpoint proteins are also needed, it seems, to re-sort repair proteins from telomeres (the DNA capping the ends of chromosomes) to sites of damage[2,3], to regulate telomere length[4,5], to direct pairs of sister-chromatid homologues during meiotic recombination[6], and to regulate the synthesis of dNTPs[7]. Checkpoint mechanisms are also interesting because of their link with cancer, first shown by the relationship between mutations in the human *p53* and *ATM* genes to cancer of the organism and to checkpoint

defects at the cellular level. Such a connection now seems plausible for *BRCA1* as well[8]. And finally, we have the eternal hope that checkpoints may somehow present a therapeutic opportunity. Caffeine, a drug long known to override checkpoints in cultured cells (in millimolar concentrations, so you don't have to put down your cup of coffee), increases cellular sensitivity to DNA damage in p53-deficient cells but not in p53-positive cells, apparently by overriding the mitotic DNA-damage checkpoint (refs 9, 10 and references therein). This appears to represent what is called synthetic lethality; that is, two defects (one genetic and one drug-induced in this case) cause greater damage sensitivity than one. Such findings lend weight to the theory that a knowledge of molecular checkpoint events will identify molecular targets for therapeutic use, maybe even in our lifetime.

So, how are checkpoint proteins activated by DNA damage, and what is their response? This very biochemical question has not yet benefited from the introduction of an *in vitro*, biochemical experimental system. Thus models of checkpoint activity (Fig. 1) are forged largely from studies of genetics, cell physiology, *in vivo* biochemistry (phosphorylation of proteins) and gene sequence data, derived mostly from fission and budding yeast model systems.

Consolidating the results from these studies, and adding a dash of wishful thinking, the present view of the DNA-damage checkpoint (using fission-yeast nomenclature) is as follows. Three sets of proteins are at work. First, so-called sensor proteins (such as Rad1, Rad17 and Crb2) probably act on damage directly. We assume that this is the case because they show limited sequence homology to an exonuclease (for Rad1) and to replication factor C (for Rad17), proteins that, at least in budding yeast, seem to mediate DNA degradation *in vivo* (see refs 11, 12 for reviews). Next, these sensors activate signal-transducing protein kinases, such as Rad3, the major checkpoint kinase. *rad3* homologues have been identified in most eukaryotes, including budding yeast (*MEC1*), flies (*mei41*), and humans (*ATM* and *ATR*). The protein kinases then modify target proteins (Rad3 targets include Cds1 and Chk1) by phosphorylation. The phosphorylated targets then alter cellular activities, probably inducing delay in the cell cycle and re-sorting of proteins from telomeres.

But it is by no means certain that sensor proteins bind to damaged DNA directly, and then activate the key Rad3 protein kinase. Although sensor proteins interact physically with each other (Fig. 1), none have yet been shown to interact physically with Rad3. So are the sensors always required for Rad3 activation? This is where Edwards *et al.*'s results come in[1]. These authors investigated how DNA damage activates Rad3 *in vivo* by assessing phosphorylation of one of Rad3's substrates, Rad26. Rad26 is itself a checkpoint protein; it is not a protein kinase, but *rad26* mutants behave very similarly to *rad3* mutants. Interestingly, Rad3 and Rad26 proteins associate physically, something that cannot yet be said for Rad3 and sensor proteins. Edwards *et al.*'s key observation is simply that, after DNA damage, Rad26 becomes phosphorylated *in vivo* by Rad3 (probably directly, although this has not been confirmed *in vitro*) (Fig. 1). There is nothing too profound here — many checkpoint proteins become phosphorylated by Rad3 after damage. Unexpectedly, however, Edwards *et al.* also show that none of the putative sensor proteins (such as Rad1) are required for Rad3 to phosphorylate Rad26; Rad3 activation appears to be unassisted by sensor proteins. In contrast, phosphorylation of other targets (Cds1 and Chk1) by Rad3 does require the assistance of the sensor proteins.

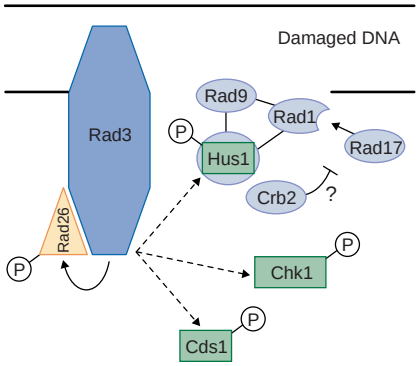

Figure 1 **Activation of the DNA-damage-repair checkpoint proteins. After DNA damage, the protein kinase Rad3 phosphorylates the target proteins Chk1, Cds1, Hus1 and Rad26. Of the sensor proteins (ovals), Rad17 may act to allow a Rad1–Rad9–Hus1 complex to recognize DNA damage. These four proteins, together with Crb2, allow Rad3 to phosphorylate some target proteins (Chk1, Cds1 and Hus1; rectangles); however, phosphorylation of another Rad3 target (Rad26; triangle) does not require Rad17, Rad1, Rad9, Hus1 or Crb2. It is largely unknown how this combination of DNA damage, sensor proteins, protein kinase and target proteins achieves cell-cycle arrest, and the relationship between the protein kinase, sensors and target proteins is uncertain. The relationships between DNA damage and these three classes of checkpoint proteins (kinase, sensors and targets) are similar in fission and budding yeast. The putative pairs of homologues are (fission yeast/budding yeast): Crb2/Rad9, Rad17/Rad24, Rad1/Rad17, Rad9/Ddc1, Hus1/Mec3, Rad3/Mec1 and Cds1/Rad53.**

Many details of this checkpoint are still unclear. For example, the consequences of Rad26 phosphorylation are unknown; it is not even certain whether its phosphorylation is required for cell-cycle arrest. Rad26 phosphorylation is certainly not sufficient for arrest, for which all of the sensor proteins shown in Fig. 1 need to be intact. In addition, sensor proteins do assist Rad3 phosphorylation of Rad26 in certain settings (when DNA replication is stalled), although not in others (after damage). Finally, what is Rad26's function? As it binds Rad3 directly, might it too act as a 'sensor' for Rad3 activation by DNA damage, as well as being a substrate for Rad3 phosphorylation?

News and Views contributions

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As a general guideline, News and Views pieces should be about 1,300 words, with one or two display items (figures, boxes and tables). They should make clear the advance (the 'news') and communicate a sense of excitement, yet provide a critical evaluation of the work in the context of the rest of the field. We encourage personal 'views', criticisms and predictions, but authors should not refer to their own work, except in passing.

Detailed guidelines are available on request from cellbio@nature.com and on *Nature Cell Biology*'s Web site (http://cellbio.nature.com).

However, despite these reservations, one fact is clear from Edwards *et al.*'s results: activation of Rad3 can be induced by DNA damage in the absence of the usual sensor proteins (Rad9, Rad1, Hus1 and Rad17). In other words, Rad3 may sense and become activated directly by DNA damage. Support for such a proposal comes from several fields of study. Damage-induced phosphorylation of some target proteins by Mec1 (the budding-yeast Rad3 homologue) requires all of the sensor proteins, yet phosphorylation of two target proteins requires only a subset of the sensors (and different subsets, as it turns out[13]). In addition, several biochemical studies of the human Rad3 homologue ATM have shown that it can be activated by added DNA damage *in vitro*[14]. However, the absence of sensor proteins in these ATM preparations has not been confirmed. Unassisted activation of the Rad3/Mec1/ATM class of protein kinase (now called 'PIKLs', for phosphatidylinositol-OH-kinase-like proteins[15]) gains further credibility from biochemical studies of the DNA-dependent protein kinase (DNA-PK). The catalytic subunit of DNA-PK can be activated by DNA damage *in vitro* in the absence of its 'sensor' protein, Ku, which binds to damaged DNA directly. DNA-PK activation by DNA damage is more robust in the presence of Ku, however, and other features distinguish the DNA-PK catalytic subunit and Ku from Rad3 and its sensor proteins; for example, the DNA-PK catalytic subunit does bind Ku, and mutations in Ku and the catalytic subunit produce similar phenotypes (ref. 15 and references therein).

The mechanism of activation of a checkpoint protein kinase is a biochemical question that clearly cannot be answered at this early stage in the game. Many issues still need to be resolved. Why does Rad3 need sensor proteins to phosphorylate some targets, but not others? What is the function(s) of sensor proteins if not to activate Rad3? Edwards *et al.* propose several explanations, and one can imagine several more.

For example, unassisted activation may achieve low levels of Rad3 kinase activity that are sufficient for phosphorylation only of tightly associated proteins, such as Rad26. In this model, sensor proteins may further activate the Rad3 kinase, perhaps by direct interaction with Rad3 or indirectly by generation of 'activating' single-stranded DNA (produced by DNA degradation and/or other mechanisms).

Alternatively, damage may indeed activate Rad3 unassisted. But what then becomes of the sensor proteins? It is possible that they may act as a 'homing site' for targets of Rad3. Thus, sensor proteins may subserve Rad3 in the same way that proteins known as 'AKAPs' assist cyclic-AMP-dependent protein kinase, that is, by recruiting the kinase to its substrates[16]. It is interesting that, in budding yeast, the sensor protein Rad9 physically interacts (in two-hybrid and co-immunoprecipitation studies) with a Mec1 target, Rad53 (ref. 17), and that Rad9 is needed for Mec1 to phosphorylate Rad53. Rad9 may have several functions, one of which is to select targets for Mec1.

Edwards *et al.*'s results[1] prompt the question of how DNA damage, sensor proteins and Rad3-type protein kinases interact. The answers will represent a substantial advance in the study of checkpoints. □

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The dynamic organizer

Ronen Schweitzer and Clifford J. Tabin

The organizer is a region in gastrulating embryos that induces and patterns the body axis. New research shows that induction of organizer formation is regulated by interplay of inducing and inhibiting ligands that affect the cells as they migrate through the organizer.

Discovery of the gastrula organizer by Spemann and Mangold 75 years ago marked a turning point in developmental biology[1,2]. The ability of this small group of cells to induce surrounding cells to form an entire embryological entity captivated the imagination of developmental biologists, and inspired numerous attempts to identify other organizing centres. The effect of the gastrula organizer is particularly dramatic: when transplanted to an ectopic site it can induce the formation of an entire new embryo. As the prototypic organizing centre, it is frequently referred to simply as 'the organizer'. Now, an exciting paper by Joubin and Stern[3] in a recent issue of *Cell* enhances our understanding of how organizer formation is induced while it uncovers some unexpected dynamic properties of the chick organizer.

After the initial discovery of the gastrula organizer, intense effort was directed towards analysis of the early events that lead to its formation, mainly in the frog *Xenopus laevis*[1,2]. Results of classical experiments suggested a two-step model. First, rotation of the cortical cytoplasm after fertilization of a *Xenopus* egg results in the formation of an organizer-inducing centre, better known as the 'Nieuwkoop centre', in the dorso-vegetal blastomeres of the early blastula. These cells subsequently induce the overlying equatorial cells to form the actual organizer.

At the molecular level, it was found that a combined action of the transforming growth factor-β (TGF-β) and Wnt pathways is required to induce the organizer. The current molecular model holds that TGF-β signalling is activated, by Vg1 or activin, TGF-β-related proteins, throughout the vegetal hemisphere of the embryo. In contrast, the Wnt cascade is activated in the absence of an extracellular signal. During cortical rotation, the β-catenin protein, a cytoplasmic mediator of the Wnt cascade, is stabilized through an unknown mechanism in a broad dorsal domain. As shown for Wnt signalling under normal circumstances, accumulation of β-catenin leads to it translocating to the nucleus and to the activation of Wnt target genes in dorsal nuclei.

A few direct Wnt targets have been identified. Of prime importance in organizer